TITLE: GLYCEROL SUPPLEMENTATION ENHANCES *LACTOBACILLUS REUTERI*'S PROTECTIVE EFFECT AGAINST *SALMONELLA* TYPHIMURIUM INFECTION IN THE COLON

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ABSTRACT:
Lactobacillus reuteri's probiotic effects have been speculated to partly depend on its capacity to convert glycerol, commonly present in feces as food or pharmaceutical residual, to the antimicrobial reuterin. In this study we investigated the potential of this process to protect human intestinal cells against infection by the enteric pathogen *Salmonella* Typhimurium.
We used a highly differentiated model of human colonic epithelium, which was previously found to reflect key characteristics of the *in vivo Salmonella* infection process. This model was infected with *S.* Typhimurium (1) in the presence of an established *L. reuteri* community without or with glycerol, or (2) under continuous exposure to the sterile *L. reuteri* ferment without and with reuterin (produced from glycerol) or pure reuterin.
Using this model, glycerol supplementation was found to significantly enhance the protective effect of an established *L. reuteri* population against the early stages of *Salmonella* infection. We further demonstrated that continuous exposure to the reuterin-containing glycerol ferment of *L. reuteri* or pure reuterin caused a significant reduction of 1-2 log units in *Salmonella* adherence, invasion and intracellular survival. Exposure to the *L. reuteri* ferment without glycerol significantly stimulated intracellular *Salmonella* growth with 1 log-unit. These data demonstrate the importance of glycerol and reuterin for *L. reuteri*'s effects on pathogen survival. However, long-term exposure (24h) to pure reuterin or the reuterin-containing ferment induced a complete loss of cell-cell contact within the epithelial structure and compromised cell viability.
Collectively, these results shed light on a biological role for reuterin in inhibiting intestinal *Salmonella* infection and support the combined application of glycerol and *L. reuteri* in reducing intestinal *Salmonella* infection, dependent upon future *in vivo* studies of reuterin on intestinal health. To our knowledge, this is the first report of the effect of reuterin on the enteric infection process in any mammalian cell type.